

CLAIMS

1. A method for purifying or capturing a non-immunoglobulin protein of interest having between one and ten immunoglobulin-like (Ig-like) domains from a biological fluid, comprising the steps of:
 - 5 a) contacting the biological fluid containing the protein of interest with an Hydrophobic Charge Chromatography (HCIC) resin,
 - b) washing out the resin to remove unbound contaminants,
 - c) eluting the protein of interest by treating the resin with a solution having an acidic pH or with a solution comprising an organic solvent.
- 10 2. A method according to claim 1, wherein the HCIC resin used in step a) is MEP-HyperCel.
3. A method according to claims 1 or 2, wherein the organic solvent used in step c) is propylene glycol.
- 15 4. A method according to claim 3, wherein the concentration of propylene glycol in the solution is between about 25 and 50%.
5. A method according to anyone of the preceding claims, wherein step a) is carried out at acidic pH.
6. A method according to claim 5, wherein the pH used is between about 3 and 6.8.
- 20 7. A method according to anyone of the preceding claims, wherein the washing of step b) is carried out with a solution having an acidic pH.
8. A method according to claim 7, wherein the pH used is between about 3 and 6.8.
9. A method according to anyone of the preceding claims wherein the biological fluid is selected from a cell-conditioned culture medium, cell lysate, cell extract, tissue extract, blood plasma, serum, milk, urine, ascites, cerebrospinal fluid, vegetable juice, plant extracts or a fraction derived from an earlier chromatographic separation step.
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10. A method according to anyone of the preceding claims, wherein the protein of interest has 1 to 7 Ig-like domains.
11. A method according to anyone of the preceding claims, wherein the protein of interest is selected from IL-18BP, NCAM , Fibronectin type III, ICAM-1, mad
5 CAM-1, PE CAM-1, VCAM-1, titin, cadherin, neurocan, LIFR, CNTFR, IL-1R, IL-3R, IL5R, IL-6R, IL-12R, GM-CSFR, OSMR, VEGF receptor , FGF receptor, hPDGF receptor, T cell receptor, MHC proteins, microglobulin- β , CTLA4, B7 activation agent, neuregulin, coagulation factor XIII, NF- κ B, IL6-IL6R, beta-galactosidase and superoxide dismutase or an isoform, mutein, fused protein, functional derivative or fragment thereof comprising at least one Ig-like domain.
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12. A method according to claim 11, wherein the protein is IL-18 binding protein (IL-18BP) .
13. A method according to claim 11, wherein the protein is IL6-IL6R chimera.
14. A method according to claim 11, wherein the protein is beta galactosidase.
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15. A method according to anyone of the preceding claims, wherein the purification factor of the eluted protein is in the range of 11 and 94 fold.
16. A method according to claim 15, wherein the purification factor of the eluted protein is about 94 fold.
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17. A method according to anyone of the preceding claims, wherein the concentration factor of the eluted protein is in the range of 1.5 and 3.1 fold.
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18. A method according to claim 17, wherein the concentration factor of the eluted protein is about 3.1 fold.
19. A method according to anyone of claims 1 to 18, wherein the yield of the eluted protein is in the range of 73 and 98%,
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20. A method according to claim 19, wherein the yield of the eluted protein is about 85%.
21. The use of a hydrophobic charge chromatography (HCIC) resin for capturing a non-immunoglobulin protein of interest having between 1 and 10

immunoglobulin-like (Ig-like) domains from a biological fluid, comprising the steps of:

- a) contacting the biological fluid containing the protein of interest with an HCIC resin,
- 5 b) washing out the resin to remove unbound contaminants,
- c) eluting the protein of interest by treating the resin with a solution having an acidic pH or with a solution comprising an organic solvent.

22. The use according to claim 21, wherein the HCIC resin used in step a) is MEP-HyperCel.

10 23. The use according to claims 21 or 22, wherein the organic solvent used in step c) is propylene glycol.

24. The use according to claim 23, wherein the concentration of propylene glycol in the solution is between about 25 and 50%.

15 25. The use according to anyone of claims 21 to 24, wherein step a) is carried out at acidic pH.

26. The use according to claim 25, wherein the pH used is between about 3 and 6.8.

27. The use according to anyone of claims 21 to 26, wherein the washing of step b) is carried out with a solution having an acidic pH.

28. The use according to claim 27, wherein the pH used is between about 3 and 6.8.

20 29. The use according to anyone of claims 21 to 28, wherein the biological fluid is selected from a cell-conditioned culture medium, cell lysate, cell extract, tissue extract, blood plasma, serum, milk, urine, ascites, cerebrospinal fluid, vegetable juice, plant extracts or a fraction derived from an earlier chromatographic separation step.

25 30. The use according to anyone of claims 21 to 29, wherein the protein of interest has 1 to 7 Ig-like domains.

31. The use according to anyone of claims 21 to 30, wherein the protein of interest is selected from IL-18BP, NCAM, Fibronectin type III, ICAM-1, mad CAM-1,

PE CAM-1, VCAM-1, titin, cadherin, neurocan, LIFR, CNTFR, IL-1R, IL-3R, IL5R, IL-6R, IL-12R, GM-CSFR, OSMR, VEGF receptor , FGF receptor, hPDGF receptor, T cell receptor, MHC proteins, microglobulin- β , CTLA4, B7 activation agent, neuregulin, coagulation factor XIII, NF-kB, IL6-IL6R, beta-galactosidase and superoxide dismutase or an isoform, mutein, fused protein, functional derivative or fragment thereof comprising at least one Ig-like domain.

- 5 32. The use according to claim 31, wherein the protein is IL-18 binding protein (IL-18BP).
- 10 33. The use according to claim 31, wherein protein is IL6-IL6R chimera.
- 10 34. The use according to claim 31, wherein the protein is beta galactosidase.
- 10 35. The use according to anyone of claims 21 to 34, wherein the purification factor of the eluted protein is in the range of 11 and 94 fold.
- 15 36. The use according to claim 35, wherein the purification factor of the eluted protein is about 94 fold.
- 15 37. The use according to anyone of claims 21 to 36, wherein the concentration factor of the eluted protein is in the range of 1.5 and 3.1 fold.
- 15 38. The use according to claim 37, wherein the concentration factor of the eluted protein is about 3.1 fold.
- 20 39. The use according to anyone of claims 21 to 38, wherein the yield of the eluted protein is in the range of 73 and 98%,
- 20 40. The use according to claim 39, wherein the yield of the eluted protein is about 85%.
- 25 41. A purified protein preparation comprising a non-immunoglobulin protein of interest having between 1 and 10 immunoglobulin-like (Ig-like) domains, purified or captured from a biological fluid by the method according to any of claims 1 to 20.
- 25 42. A purified protein preparation according to claim 41, wherein the protein of interest is selected from IL-18BP, NCAM , Fibronectin type III, ICAM-1, mad

CAM-1, PE CAM-1, VCAM-1, titin, cadherin, neurocan, LIFR, CNTFR, IL-1R, IL-3R, IL5R, IL-6R, IL-12R, GM-CSFR, OSMR, VEGF receptor , FGF receptor, hPDGF receptor, T cell receptor, MHC proteins, microglobulin- β , CTLA4, B7 activation agent, neuregulin, coagulation factor XIII, NF-kB, IL6-IL6R, beta-galactosidase and superoxide dismutase or an isoform, mutein, fused protein, functional derivative or fragment thereof comprising at least one Ig-like domain.

- 5 43. A protein preparation according to claim 42, wherein the protein is IL-18BP.
44. A protein preparation according to claim 42, wherein the protein is IL6-IL6R.
- 10 45. A protein preparation according to claim 42, wherein the protein is beta galactosidase.

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